App No.: 10/512,028 Docket No.: 506612000103

Inventor: Jay WOHLGEMUTH et al.
Title: METHODS AND COMPOSITIONS FOR DIAGNOSING
AND MONITORING AUTO IMMUNE AND CHRONIC, etc.
REPLACEMENT SHEET
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Figure 3: Kits for discovery of, or application of diagnostic gene sets

A. Contents of kit for discovery of diagnostic gene sets using microarrays

- 1. Sterile, endotoxin and RNAse free blood collection tubes
- 2. Alcohol swabs, tourniquet, blood collection set
- 3.-PBS (phosphate buffer saline; needed when method of example 8 is used to derived mononuclear RNA)
- 4. Cell lysis buffer
- 5. RNA isolation kit
- 6. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence microarray expression profiling:

Reverse transcriptase and 10x RT buffer

T7(dT)24 primer (primer with T7 promoter at 5' end)

DTT

Deoxynucleotides 100mM each

RNAse inhibitor

2nd strand cDNA buffer

DNA polymerase

Rnase H

T7 RNA polymerase

Ribonucleotides

In Vitro transcription buffer

- Cv3 and Cv5 labeled ribonucleotides
- 7. Microarrays containing candidate gene libraries
- 8. Cover slips for slides
- 9. Hybridization chambers
- 10. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database server

- 11. Password and account number to access central database server.
- 12. Kit User Manual

B. Contents of kit for application of diagnostic gene sets using microarrays

- 1. Sterile, endotoxin and RNAse free blood collection tubes
- 2. Alcohol swabs, tourniquet, blood collection set
- 3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)

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- 4. Cell lysis buffer
- 5. RNA isolation kit
- 6. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence microarray expression profiling:

Reverse transcriptase and 10x RT buffer

T7(dT)24 primer (primer with T7 promoter at 5' end)

DTT

Deoxynucleotides 100mM each

RNAse inhibitor

2nd strand cDNA buffer

DNA polymerase

Rnase H

T7 RNA polymerase

Ribonucleotides

In Vitro transcription buffer

Cv3 and Cv5 labeled ribonucleotides

- 7. Microarrays containing candidate gene libraries
- Cover slips for slides
- Hybridization chambers
- 10. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database

- 11. Password and account number to access central database server.
- 12. Kit User Manual

C. Contents of kit for application of diagnostic gene sets using Real-time RT-PCR

- 1. Sterile, endotoxin and RNAse free blood collection tubes
- 2. Alcohol swabs, tourniquet, blood collection set
- 3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
- 4. Cell lysis buffer
- 5. RNA isolation kit
- 6. Substrates for real time RT-PCR (may vary for various real-time PCR techniques:

poly dT primers, random hexamer primers

Reverse Transcriptase and RT buffer

DTT

Deoxynucleotides 100 mM

RNase H

primer pairs for diagnostic and control gene set

10x PCR reaction buffer

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Taq DNA polymerase

Fluorescent probes for diagnostic and control gene set

(alternatively, fluorescent dye that binds to only double stranded DNA)

reaction tubes with or without barcode for sample tracking

96-well plates with barcode for sample identification, one barcode for entire set, or individual barcode per reaction tube in plate

7. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set. Software facilitates access to and data analysis by centrally located database server

- 8. Password and account number to access central database server.
- 9. Kit User Manual